

## **EXHIBIT 3**

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(56) Documents Cited

None

(58) Field of Search

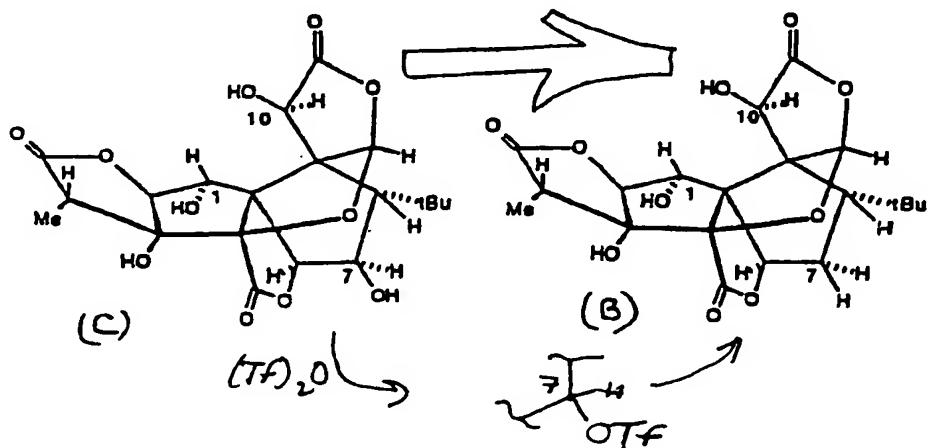
UK CL (Edition N) C2C CAP CTU

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Online databases: CAS-ON-LINE

## (54) Conversion of ginkgolide C to ginkgolide B

(57) A two-step process for converting ginkgolide C into ginkgolide B is disclosed. In the first step, ginkgolide C is reacted with a sulphonic anhydride to obtain 7-sulphonyloxy-ginkgolide B. In the second step, the 7-sulphonyloxy-ginkgolide B is reacted with a borohydride to eliminate the 7-sulphonyloxy group and thus produce ginkgolide B.



GB 2 288 599 A

Applicants: Koji Nakanishi et al.

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Exhibit 3

TITLE:

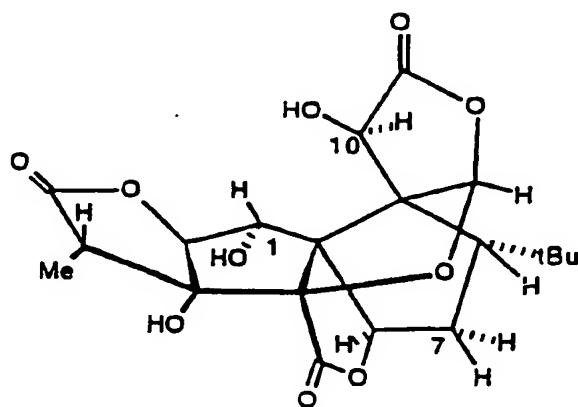
Conversion of Ginkgolide C to Ginkgolide B

DESCRIPTION:

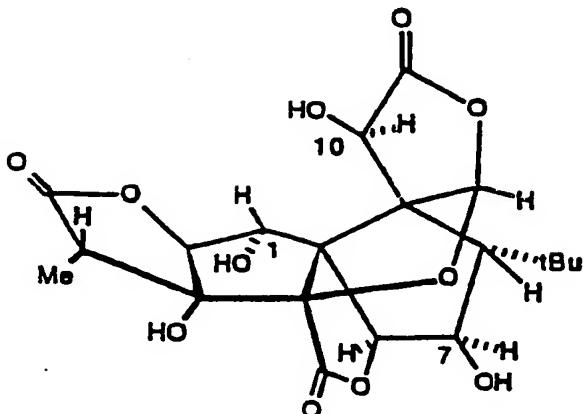
The invention relates to a process for the conversion of ginkgolide C into ginkgolide B.

Ginkgolides are diterpene lactones which are isolated from leaves and roots of the ginkgo biloba tree. Ginkgolides B and C are the predominant ginkgolides extracted, and they are extracted in approximately equal quantities. Ginkgolide B has been found to be a potent inhibitor of PAF-acether, see for example U.S. Patent 4734280. Ginkgolide C has some activity, but is not nearly as active as ginkgolide B. The extraction of the ginkgolides is a costly process. As far as the applicant knows, there is no way selectively to extract only ginkgolide B; all of the ginkgolides are extracted and then are isolated from each other. Therefore, a process for converting ginkgolide C to ginkgolide B is of definite interest.

Ginkgolides B and C are quite similar chemically. The chemical formula of ginkgolide B is as follows:



The chemical formula of ginkgolide C is as follows:



As can be seen, the only difference between the two is that one of the hydrogen atoms at the 7-position of ginkgolide B is replaced by a hydroxy group in ginkgolide C.

U.S. Patent 5241084 discloses a process for converting ginkgolide C to ginkgolide B. The process is a rather lengthy four step process and consists of the following four successive steps:

- protecting the 10-hydroxy group of ginkgolide C by conversion to an alkyl ester, the reaction being effected in dimethylformamide at a temperature of from 15° to 50°C for from 4 to 10 hours;

- activating the 7-hydroxy group of the resultant 10-protected ginkgolide C by conversion to a (R)thiocarbonyl ester, the activation being effected in basic conditions at a temperature of from 0°C to 40°C for from 1 to 24 hours;

- deoxygenating the 7-activated group in the resultant 10-protected 7-activated ginkgolide C by treating it with tributyltin hydride or tris-(trimethyl-

silyl)silane, in an aprotic solvent, in the presence of a free-radical generator, the reaction being effected at a temperature of from 70° to 110°C for from 15 minutes to 3 hours under inert atmosphere; and

- cleaving the protecting group from the 10-hydroxy group of the resultant 10-protected ginkgolide B.

While the process of US 5241084 is very valuable in that the conversion of ginkgolide C to ginkgolide B by the four step process is much less expensive than the extraction of ginkgolide B from the ginkgo tree, a simpler process would be even more valuable.

The applicant has now discovered a process for converting ginkgolide C to ginkgolide B in only two steps. The applicant's two step process is as follows:

- reacting ginkgolide C with a sulphonic anhydride to obtain a 7-sulphonyloxy-ginkgolide B, the reaction preferably being effected in a basic medium at a temperature of from about -20°C to about 35°C and for a period of from about 15 minutes to about 3 hours; and

- reacting the 7-sulphonyloxy-ginkgolide B with a borohydride in an aprotic solvent, the reaction preferably being effected at a temperature of from about 10°C to about 30°C and for a period of from about 15 minutes to about 3 hours, the reaction eliminating the 7-sulphonyloxy group and thus producing ginkgolide B.

The first step of the two step process is carried out in a basic medium. The solvent used is preferably pyridine, a mixture of pyridine and dichloromethane, a mixture of acetonitrile and triethylamine, 4-dimethylaminopyridine or imidazole.

Sulphonic anhydrides suitable for use in the first step of the process of the invention may be characterized as R-sulphonic anhydrides in which R represents a halogen atom, a C<sub>1</sub>-C<sub>6</sub> alkyl group, a halogen substituted C<sub>1</sub>-C<sub>6</sub> alkyl group, a phenyl group or a substituted phenyl group. Preferred values for R are methyl, n-butyl, trifluoromethyl, toluyl, p-nitrophenyl, p-bromophenyl and 2,4,6-trinitrophenyl groups.

It is most preferred that the reaction be carried out in pyridine using from 1 to 4 equivalents of the trifluoromethanesulphonic anhydride.

Borohydrides suitable for use in the second step of the process of the invention may be characterized as R'-borohydrides wherein R' may be an alkali metal or an ammonium group. The preferred alkali metal borohydride is sodium borohydride. Suitable ammonium borohydrides are tetraarylalkylammonium borohydride and tetraalkylammonium borohydride wherein the alkyl groups can have from 1 to 6 carbons and the aryl groups may be monocyclic or bicyclic. The preferred ammonium borohydride is tetrabutylammonium borohydride. The second step is preferably carried out in tetrahydrofuran (THF).

The following Examples illustrate the invention.

EXAMPLE 1

Step 1: preparation of 7-trifluoromethanesulphonyloxy-ginkgolide B

11.4 ml of trifluoromethanesulphonic anhydride was added at a temperature below -10°C to a solution of 6 ml of pyridine in 150 ml of dichloromethane which had been cooled to -15°C. A solution of 26.4 g of ginkgolide C in 225 ml of pyridine was added at the same temperature. The mixture was stirred for 2 hours at -15°C and the

temperature was then allowed to rise to room temperature. The mixture was concentrated under reduced pressure and the residue was treated with 150 ml of ethyl acetate. The obtained solution was washed with 75 ml of 1N hydrochloric acid, then twice with 75 ml of sodium chloride solution. The organic phase was treated with activated carbon (3%), dried over anhydrous magnesium sulphate and concentrated under vacuum. The resultant residue was treated with a mixture of methyl t-butyl ether and heptane (250/520 ml). The suspension was filtered and the solid was washed twice with 50 ml of heptane. Yield 91%.

Step 2: preparation of ginkgolide B

A solution of 11.47 mg of tetrabutylammonium borohydride in 50 ml of THF was added dropwise at 20°C to a solution of 25.5 g of 7-trifluoromethanesulphonyloxy-ginkgolide B (prepared as described in Step 1 above) in 200 ml of THF. The reaction mixture was stirred for 1 hour at 20°C, and then cooled to 10°C and treated with 250 ml of methanol. The solution was concentrated and the residue was treated with 150 ml of ethyl acetate. The mixture thus obtained was washed successively with 60 ml of ammonia solution, 60 ml of 20% sodium chloride solution, 60 ml of 1N hydrochloric acid and 15 ml of sodium chloride solution. The solvent was eliminated under reduced pressure and the obtained residue was treated in the hot with a water:ethanol mixture (300:100 ml). The resultant solution was cooled and the product recrystallised. The product was filtered off, washed and then dried. Yield 85.8%.

EXAMPLE 2

Example 1 was repeated except that methanesulphonic anhydride was used instead of trifluoromethanesulphonic anhydride in Step 1. The yield from the first step was 90.5%. The yield from the second step which was carried

out in the manner set forth in Example 1 was 84%.

EXAMPLE 3

Example 1 Step 1 was repeated except that n-butane-sulphonic anhydride was used instead of trifluoromethane-sulphonic anhydride. The yield from the first step was 87%. In the second step, sodium borohydride was substituted for the tetrabutylammonium borohydride. The yield of ginkgolide B was 80.2%.

EXAMPLE 4

Example 1 was repeated except that benzenesulphonic anhydride was substituted for the trifluoromethane-sulphonic anhydride in the first step. The yield was 86%. After the second step, which was carried out in the manner described in Example 1, the yield of ginkgolide B was 83%.

EXAMPLE 5

Example 1 was repeated except that toluenesulphonic anhydride was used in place of trifluoromethanesulphonic anhydride in the first step. The yield after the first step was 90%. The second step, carried out as in Example 1, resulted in an 86.7% yield of ginkgolide B.

EXAMPLE 6

Example 1 was repeated except that 2,4,6-trinitrobenzene-sulphonic anhydride was used instead of trifluoromethane-sulphonic anhydride in step 1. The yield from the first step was 86%. The second step was carried out in the manner described in Example 1 and the yield was 83% of ginkgolide B.

CLAIMS

1. A process for the preparation of ginkgolide B from ginkgolide C, the process comprising the following two steps:
  - (a) reacting ginkgolide C with a sulphonic anhydride to obtain a 7-sulphonyloxy-ginkgolide B; and
  - (b) reacting the 7-sulphonyloxy-ginkgolide B with a borohydride in an aprotic solvent.
2. A process according to claim 1 wherein the reaction of step (a) is effected in a basic medium at a temperature of from about -20°C to about 35°C and for a period of from about 15 minutes to about 3 hours.
3. A process according to claim 1 or claim 2 wherein the reaction of step (b) is effected at a temperature of from about 10°C to about 30°C and for a period of from about 15 minutes to about 3 hours.
4. A process according to any preceding claim wherein the sulphonic anhydride is an R-sulphonic anhydride in which R represents a halogen atom, a C<sub>1</sub>-C<sub>6</sub> alkyl group, a halogen substituted C<sub>1</sub>-C<sub>6</sub> alkyl group, a phenyl group or a substituted phenyl group.
5. A process according to claim 4 in which R represents a methyl, n-butyl, trifluoromethyl, toluyl, p-nitrophenyl, p-bromophenyl or 2,4,6-trinitrophenyl group.
6. A process according to any preceding claim wherein the borohydride is an R-borohydride in which R represents an alkali metal or an ammonium group.
7. A process according to claim 6 in which R represents sodium.

8. A process according to claim 6 in which R represents a tetraarylalkylammonium and tetraalkylammonium group.
9. A process according to claim 8 wherein the borohydride is tetrabutylammonium borohydride.
10. A process according to any preceding claim wherein the reaction of step (a) is carried out a solvent selected from pyridine, a mixture of pyridine and dichloromethane, a mixture of acetonitrile and triethylamine, 4-dimethylaminopyridine and imidazole.
11. A process according to claim 10 wherein the reaction of step (a) is carried out in pyridine.
12. A process according to any preceding claim wherein the reaction of step (b) is carried out in tetrahydrofuran.
13. A process for the preparatin of ginkgolide B from ginkgolide C, the process being substantially as described herein with reference to any of the Examples.

**Patents Act 1977**  
**Examiner's report to the Comptroller under Section 17**  
**(The Search report)**

**Application number**  
**GB 95 901.0**

**Search Examiner**  
**S I AHMAD**

**Date of completion of Search**  
**22 MAY 1995**

**Documents considered relevant**  
**following a search in respect of**  
**Claims :-**  
**1-13**

**Databases (see below)**

(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ON-LINE DATABASE: CAS-ON-LINE

**Categories of documents**

- |    |   |     |   |
|----|---|-----|---|
| X: | Document indicating lack of novelty or of inventive step.   | P:  | Document published on or after the declared priority date but before the filing date of the present application.        |
| Y: | Document indicating lack of inventive step if combined with one or more other documents of the same category. | E:  | Patent document published on or after, but with priority date earlier than, the filing date of the present application. |
| A: | Document indicating technological background and/or state of the art.   | &c: | Member of the same patent family; corresponding document.   |

Category	Identity of document and relevant passages	Relevant to claim(s)
	NONE	

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